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Redox-responsive hyaluronic acid nanogels for hyperthermia- assisted chemotherapy to overcome multidrug resistance

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ABSTRACT

Although chemotherapy has been widely used in the treatment of many kinds of cancer, drug resistance and side effects are the main obstacles in the cancer chemotherapy that result in an inferior therapeutic outcome. For the design of drug delivery system, extracellular stability and intracellular effective release are also a pair of contradictions. In this research, gold nanorods (AuNRs) loaded hyaluronic acid (HA) nanogels with reduction sensitivity were prepared for the efficient intracellular delivery of doxorubicin (DOX). The aforementioned HA-CysNG@AuNR nanogels with cystamine (Cys) as crosslinker could remain stable in the physiological condition and release DOX rapidly in the mimic intracellular glutathione (GSH) condition. Meanwhile, the cellular uptake efficiency by the human breast carcinoma (MCF-7) cells was enhanced because of the highly expressed HA receptor (CD44) on the cytomembrane. However, further cell experiments verified that it was difficult to achieve desired results for drug-resistant human breast cancer (MCF-7 ADR) cells due to the reduced drug uptake and enhanced drug efflux. Interestingly, this multidrug resistance of MCF-7 ADR cells could be reversed after treated with near-infrared (NIR) light. This might ascribe to the hyperthermia generated by AuNRs under NIR, which suspended drug efflux process and led to excellent hyperthermia-assisted chemotherapy outcome. Overall, our studies suggested that AuNRs loaded reduction-sensitive HA nanogels were excellent candidates of drug carriers to reverse the drug-resistance and induce severe apoptosis of drug-resistant MCF-7 ADR cells.

1. Introduction

Chemotherapy is one of the most common treatments for cancer therapy. However, multiple drug resistance and side effects are main obstacles of conventional chemotherapy, which extremely limit the therapeutic efficacy(Gottesman, 2002; Pastan, 1991). For example, previous studies proved the drug resistance of doxorubicin (DOX) was often caused by reduced drug uptake and enhanced drug efflux. Thus the intracellular drug concentration was significantly reduced to suspend cell apoptosis of cancer cells (Khamisipour, Jadidi-Niaragh, Jahromi, Zandi, & Hojjat-Farsangi, 2016). Following this process, chemotherapy might result in an inferior therapeutic outcome. To reach the effective therapeutic effect, larger dose of drug was adopted, which usually caused more serious side effects to normal tissues (Holohan, Van Schaeybroeck, Longley, & Johnston, 2013).

Numerous drug delivery systems have been designed to increase the therapeutic efficacy as well as reduce the side effects (Chen et al., 2015; Dreaden, Alkilany, Huang, Murphy, & El-Sayed, 2012; Jain & Stylianopoulos, 2010; Li, Zhang et al., 2016; Liu, Qiao, Yang, Weng, & Zhang, 2014; Riehemann et al., 2009; Song et al., 2016; Wang, Wu, Liu, Du, & Cheng, 2016). Stimuli-responsive drug delivery systems have been drawing great attention, which could facilitate the intracellular-triggered release and further reinforce tumor-killing efficacy (Cheng, Meng, Deng, Klok, & Zhong, 2013; Liu et al., 2011; Meng, Cheng, Deng, & Zhong, 2012; Wang, Liu, Dong et al., 2015; Wang, Liu, Gao, & Wang, 2015; Wang, Ren, Wang, Wang, & Ji, 2015). The glutathione (GSH) concentration (2–10 mM) inside the tumor cells is 100- to 1000-fold higher than the extracellular fluids, which provides some advantages to design the redox-responsive drug delivery systems (Cheng et al., 2011; Sun, Meng, Cheng, Deng, & Zhong, 2013). Recent years have witnessed the fast development of near-infrared (NIR) photothermal therapy in the treatment of cancer (Wu et al., 2018). Due to the deep penetration up to 4–10 cm in tissues (Weissleder, 2001), the NIR photothermal therapy could damage deeply buried cancer cells via the hyperthermia generated by the photoabsorbing agents such as cyanine dyes(Yang et al., 2013), graphene oxide (Zhang et al., 2011) and gold

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nanoparticles(Hirsch et al., 2003). Some researches indicated that the hyperthermia could enhance the cytotoxicity of the anticancer drug, which finally increased the therapeutic efficacy with the same dosage of drug (Hahn, Braun, & Har-Kedar, 1975; Johnson & Pavelec, 1973; Overgaard, 1976). In particularly, the drug resistance of tumor cells to the chemotherapeutic agents was proved to be eliminated via combination of chemotherapy effect and hyperthermia effect of photothermal therapy (Coelho et al., 2013; Coelho, Pereira, Juzeniene, Juzenas, & Coelho, 2015; Meng et al., 2016; Zhu et al., 2015). Li et al synthesized a polymeric prodrug to co-load photothermal agent IR-780 and doxorubicin (DOX), which could reverse drug resistance of tumor cells through hyperthermia-assisted site-specific chemotherapy approach (Li, Wang et al., 2016). Nonetheless, the medical application of IR-780 was greatly limited by the low optical absorption coefficient. Thus, it is still an urgent task to easily establish drug delivery systems with effective reversal of the drug resistance for successful chemotherapy.

Gold nanorods (AuNRs) are usually adopted in many photothermal therapy systems due to the excellent biocompatibility, large-scale synthesis with easy method, high photothermal conversion efficiency in the NIR region (Liu, Huang et al., 2014). In this study, the cross-linking hyaluronic acid (HA) nanogels (HA-CysNG) and AuNRs-loaded nanogels (HA-CysNG@AuNR) were easily prepared. HA could remarkably enhance the biocompatibility, stability and increase the endocytosis efficiency via specific binding the CD44 receptors overexpressed by some cancer cells (Rao et al., 2016; Wang, Wang et al., 2015, Wang, Liu, Gao et al., 2015; Wang, Ren et al., 2015). The negative-charged nanoparticles could effectively load the positive doxorubicin (DOX) through strong electrostatic interactions. The in vitro drug release experiment was conducted at the mimic intracellular GSH concentration. Cell experiments were carried out to test the hyperthermia-assisted chemotherapy effect in the drug-resistant human breast cancer (MCF-7 ADR) cells. The mechanism of drug-resistant reversal was further explored by the fluorescence microscope.

2. Experimental section

2.1. Chemicals and materials

Hyaluronic acid (HA, MW $5*10^4$ Da) was obtained from Zhenjiang Dong Yuan Biotech Co., Ltd. (China). Sodium borohydride ($NaBH_4$), 4-nitrophenyl chloroformate 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), doxorubicin (DOX) hydrochloride, glutathione (GSH) and 1-ethyl-3-[3-(dimethylamino) propyl]-carbodiimide (EDC) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Cystamine dihydrochloride (Cys) and silver nitrate ($AgNO_3$) were used as received from J&K scientific (Shanghai, China). Chloroauric acid ($HAuCl_4$), ascorbic acid (AA), cetyltrimethyl ammonium bromide (CTAB), span 80 and liquid paraffin were purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Preparation of gold nanorods (AuNRs)

Seed-mediated growth method was chosen to synthesize AuNRs(Liu, Huang et al., 2014). Firstly, 0.6 mL of 10 mM $NaBH_4$ was dissolved in ice water. The solution was added into 10 mL solution of 100 mM CTAB and 0.25 mM $HAuCl_4$ along with vigorous stirring for 2–3 min. When the color of the mixture turned into dark brown, the seed solution was obtained. Secondly, to prepare the growth solution, 1 mL of 10 mM $AgNO_3$, 0.2 mL of 1 M HCl and 0.7 mL of 78.8 mM AA was added into 100 mL solution of 100 mM CTAB and 0.5 mM $HAuCl_4$. Then 0.12 mL two hours aged seed solution was rapidly added into the growth solution and the final solution were kept at 27 °C for 14–16 h to get CTAB-capped AuNRs. After centrifuging twice at 15,000 rpm for 15 min, the synthesized AuNRs were measured by ultraviolet-visible (UV–vis) spectrum and transmission electron microscopy (TEM, JEM-1200EX, NEC, Tokyo, Japan) operated at 80 kV.

2.3. Synthesis and characterization of cross-linking HA nanogels and AuNRs-loaded nanogels

Emulsion method was used to prepare cross-linking nanogels (HA-CysNG) for the delivery of doxorubicin(Liu, Chen, Mao, & Gao, 2007). Briefly, 330 mg HA was dissolved in 10 mL of PBS (pH 7). 478 mg EDC was used to activate the carboxyl of the HA. After 2 h reaction, 93 mg cystamine was added into the solution. Then the mixture was dropped into 150 mL of 10 wt% span 80 solution in liquid paraffin with magnetic stirring at 1500 rpm. After overnight reaction, the obtained mixture was centrifuged (15,000 rpm for 5 min) and washed with petroleum ether (2 times) and ethyl alcohol (2 times). The final product was further freeze-dried for 2 days to get HA-CysNG particles.

To obtain the AuNRs-loaded nanogels (HA-CysNG@AuNR), 330 mg HA was dissolved in 9 mL of PBS (pH 7) and 478 mg EDC was used to activate the carboxyl of the HA for 2 h. Then 93 mg of Cys and 1 mL of AuNRs solution were added into the solution. In the following steps, the same method was used as above.

The fourier transform infrared spectra (FT-IR) of free HA, HA-CysNG and HA-CysNG@AuNR were performed on a Spectrometer (Spectrum Two, PerkinElmer) ranging from 400 to 4000 cm^{-1}. The morphologies of final particles were measured by TEM. The hydrodynamic size and zeta potential of products were measured by dynamic light scattering (DLS) on a Zetasizer Nano ZS90 (Malvern Inst. Ltd, UK).

2.4. Preparation and characterization of DOX-loaded nanogels

To further synthesize DOX-loaded nanogels, 50 mg HA-CysNG or 50 mg HA-CysNG@AuNR nanogels were dissolved in 5 mL PBS (pH 9), respectively. Then 1 mg DOX was added in separately. After stirring overnight, the mixture was centrifuged to remove free DOX. The supernatant was measured by UV–vis spectrometer at 490 nm to confirm the DOX encapsulation efficiency. This process was proceeded based on a DOX calibration curve, which adopted least-squares approach to fit the data. The concentrations of Au in the AuNR-loaded nanogels(HA-CysNG@AuNR and HA-CysNG@AuNR@DOX) were measured by inductively coupled plasma mass spectrometry (ICP-MS).

The morphologies of the DOX-loaded nanogels (HA-CysNG@DOX and HA-CysNG@AuNR@DOX) were also measured by TEM and the hydrodynamic size and zeta potential of products above were measured by DLS.

2.5. NIR photothermal effect of AuNRs-loaded nanogels

In order to test NIR photothermal effect of AuNRs-loaded nanogels, HA-CysNG@AuNR and HA-CysNG@AuNR@DOX nanogels were performed at the Au concentration of 0.3 μg/mL in the same volume of solution. The HA-CysNG@DOX and PBS solution were used as control. The temperature changes of each sample were measured in intervals during 180 s by a ~6 mm focused spot size with the irradiation of 0.7 $W*cm^{-2}$ continuous laser at 808 nm(Li, Zhang, Du, Zhao, & Wang, 2017).

2.6. In vitro drug release studies

To study the redox-responsive release process of DOX *in vitro*, 1 mg HA-CysNG@DOX and 1 mg HA-CysNG@AuNR@DOX nanogels were placed in 1 mL of PBS (pH 7.4) with 10 mM GSH at 37 °C respectively. The concentration of DOX and Au was 5 μg/mL of and 0.3 μg/mL respectively. The same samples were placed in 1 mL of PBS without GSH as control. The solutions were centrifuged at 12,000 rpm for 5 min at 0.5 h, 1 h, 2 h, 4 h respectively and the supernatants were measured by spectrometer at 490 nm to determine DOX release data (Maciel et al., 2013).